AD_____

Award Number: W81XWH-12-1-0535

TITLE: A Multiplex Cancer/Testis Antigen-Based Biomarker Panel to Predict the Aggressive Phenotype of Prostate Cancer

PRINCIPAL INVESTIGATOR: Prakash Kulkarni

CONTRACTING ORGANIZATION: Johns Hopkins University School of Medicine
Baltimore, MD 21205

REPORT DATE: October 2013

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October-2013	Annual Report	30September2012-29September2013

4. TITLE AND SUBTITLE: A Multiplex Cancer/Testis Antigen-Based Biomarker Panel to Predict the Aggressive Phenotype of Prostate Cancer

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-12-1-0535

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Prakash Kulkarni

E-Mail: pkulkar4@jhmi.edu

5d. PROJECT NUMBER
W91ZSQ1365N635

5e. TASK NUMBER
Task #1 and #2

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Johns Hopkins University School of Medicine

Baltimore, MD 21205

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT: The Cancer/Testis Antigens (CTAs) are a unique group of proteins that are normally confined to germ cells but aberrantly expressed in several types of cancers. Several studies have shown that their expression patterns are frequently associated with higher grade lesions and advanced disease with a poorer outcome. The central hypothesis of this grant application is that a CTA-based biomarker can be used to discern PCa patients with aggressive disease (high likely hood of recurrence following RP, presentation of advanced disease and/or cancer specific mortality) and hence would need definitive treatment from those in whom it is less likely to recur (less likely to recur post RP or advance) and would therefore, not require immediate intervention. In the first year of this grant our goal was to accomplish Specific Aim namely, identify candidate CTAs that are differentially expressed in clinically organ-confined PCa and metastatic PCa tissues using the NanoString technology. The results indicate that NanoString nCounter analysis is an appropriate platform to analyze CTAs expression in samples from PCa patients and CTAs can be used to differentiate metastatic PCa from localized PCa.

15. SUBJECT TERMS none provided

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU		19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

Introduction

Prostate cancer (PCa) is the most commonly diagnosed cancer and the second leading cause of cancer-related deaths in men in the United States. Early detection of PCa is aided by the blood test PSA and patients with definitive diagnoses typically undergo what is considered to be successful therapy for their localized disease. However, in a small percentage of these men, the disease recurs and is often then treated with therapies focused on blocking androgens and androgen action. The Cancer/Testis Antigens (CTAs) are a unique group of proteins that are normally confined to germ cells but aberrantly expressed in several types of cancers. Several studies have shown that their expression patterns are frequently associated with higher grade lesions and advanced disease with a poorer outcome. The central hypothesis of this grant application is that a CTA-based biomarker can be used to discern PCa patients with aggressive disease (high likely hood of recurrence following RP, presentation of advanced disease and/or cancer specific mortality) and hence would need definitive treatment from those in whom it is less likely to recur (less likely to recur post RP or advance) and would therefore, not require immediate intervention. This hypothesis will be addressed with the following specific aims: 1) to identify candidate CTAs that are differentially expressed in clinically organ-confined PCa and metastatic PCa tissues; 2) to develop the expression profile of CTAs to predict the aggressiveness of PCa using the CTA-based nCounter Gene Expression Assay; and 3) To test whether the CTAs expression profiling can differentiate the 'aggressive' versus 'indolent' PCa using blinded samples. In addition to the blinded sample sets, this specific aim will also explore the possibility of using peripheral blood cells that contain circulating tumor cells for assaying the CTAs expression profiling.

Body

Clinical samples

Samples from clinically localized prostate cancer (n=20) and soft tissue metastasis (n=20) were obtained at University of Washington. The age range of the patients with clinically localized prostate cancer was 48‑75 years (median 58 years) and a preoperative serum PSA was a median of 7.54 (ng/ml) (range, 2.4 to 64.0). The GS was: 6 (n=3), 7 (n=14), 8 (n=1) and 9 (n=2), respectively. Soft tissue metastasis were obtained from lymph node (n=8), liver (n=5), adrenal (n=1), bladder (n=1), kidney (n=1), lung (n=1) and pancreas (n=1), respectively. The specimens were used with the approval of the University of Washington Institutional Review Boards.

RNA extraction

Total RNA was isolated using the miRNeasy Kit according to the manufacturer's instructions (Qiagen, Valencia, CA). RNA integrity for each sample was confirmed with the Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA).

NanoString nCounter analysis

Nanostring nCounter analysis was performed using a custom-designed codeset containing 22 CTA genes according to the manufacturer's protocol (NanoString Technologies, Seattle, WA). Each reaction contained 250 ng of total RNA in a 5 uL aliquot, plus reporter and capture probes, and 6 pairs of positive control and 8 pairs of negative control probes. All the process was performed at the Johns Hopkins Deep Sequencing & Microarray Core Facility. Raw counts were normalized to internal mRNA levels of the β-actin gene.

Quantitative real-time PCR

First strand cDNA was made from 1 µg RNA using iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Inc., Hercules, CA) following the manufacturer's protocol in a total volume of 20 µl. Quantitative real-time PCR (Q-PCR) were carried out as previously described (20). In brief, the PCR reactions were performed with 0.2 µl of cDNA template in 25 µl of reaction mixture containing 12.5µl of iQ SYBR Green Supermix (Bio-Rad Laboratories, Inc.) and 0.25 µmol/L each primer. PCR reactions were subjected to hot start at 95^{o}C for 3 minutes followed by 45 cycles of denaturation at 95^{o}C for 10 seconds, annealing at 60^{o}C for 30 seconds, and

extension at 72^{o}C for 30 seconds using the CFX96 Real-Time PCR Detection System (Bio-Rad Laboratories, Inc.). Analysis and fold differences were determined using the comparative threshold cycle method. All experiments were performed in triplicate and data presented represents mean ± SD.

Results

We first selected 22 Cancer/Testis Antigens (CTAs) according to our previously obtained microarray data using CT Array v2 that we developed in combination with data from Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo). Signals of all the 22 CTAs were successfully detected by NanoString nCounter analysis in samples from metastatic and localized prostate cancer (PCa). Out of these 22 CTAs, at least 10 CTAs (CEP55, CSAG2, MAGEA1, MAGEA6, MAGEA12, NUF2, PAGE4, PBK, SPAG4, TTK) were differentially expressed between metastatic PCa and localized PCa. Representative results were shown in Figure 1. CEP55, PBK and NUF2 were up-regulated and PAGE4 was down-regulated in metastatic PCa compared to localized PCa (Fig. 1). Furthermore, these expression data obtained from NanoString nCounter analysis were validated by Q-PCR (Fig. 1). These results indicate that NanoString nCounter analysis is an appropriate platform to analyze CTAs expression in samples from PCa patients and CTAs can be used to differentiate metastatic PCa from localized PCa.

Key Research Accomplishments

- Signals of all the 22 CTAs were successfully detected by NanoString nCounter analysis in samples from metastatic and localized prostate cancer (PCa).
- 10 CTAs were differentially expressed between metastatic PCa and localized PCa.
- Expression data obtained from NanoString nCounter analysis were validated by Q-PCR
- CTAs expression can be used to differentiate metastatic PCa from localized PCa
- NanoString nCounter analysis is an appropriate platform to analyze CTAs expression in samples from PCa patients

Reportable Outcomes

None

Conclusions

References

None

Appendices

None

Supporting data

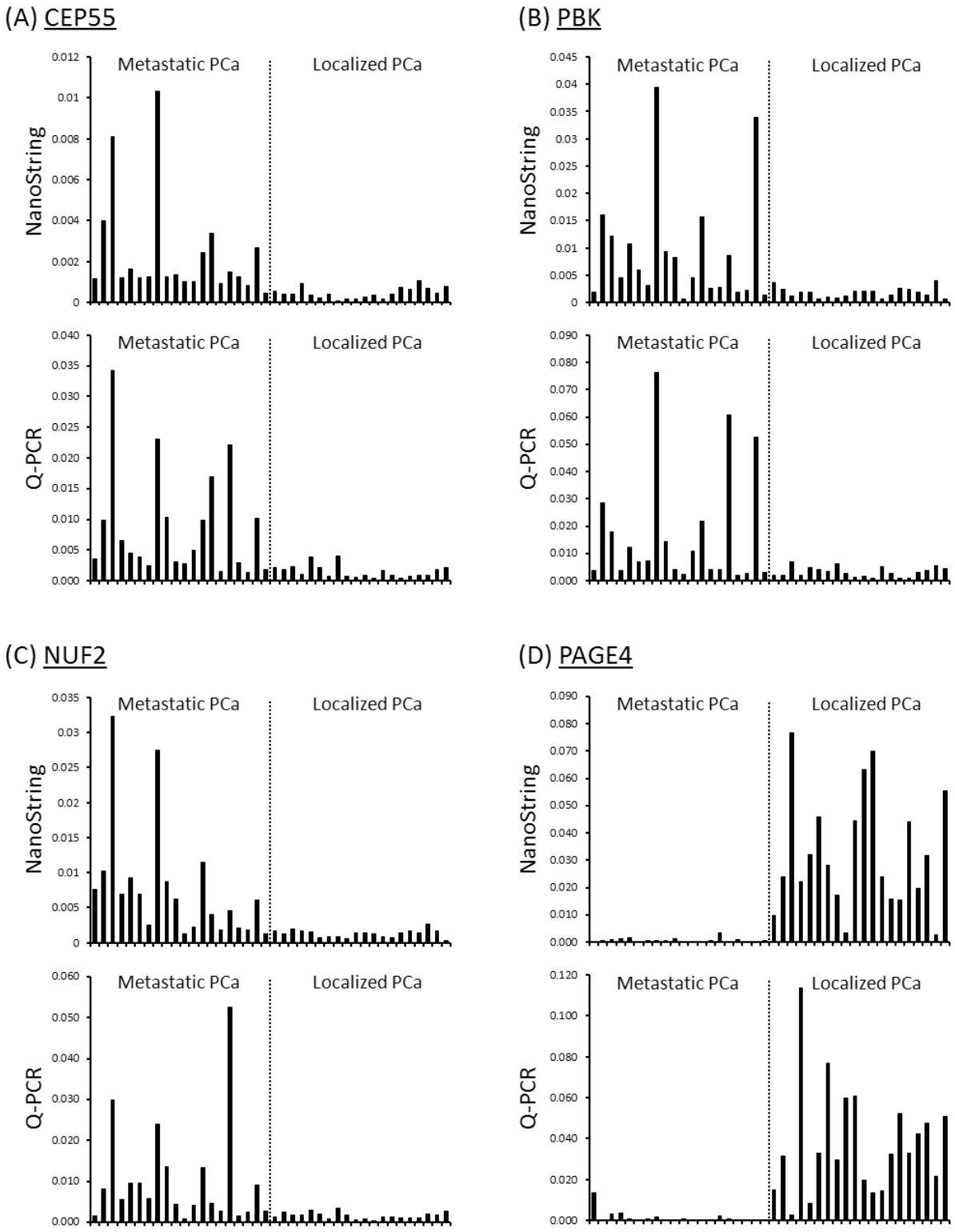

Figure 1. Representative CTAs expression in metastatic and localized prostate cancer
CEP55 (A), PBK (B), NUF2 (C) and PAGE4 (D) mRNA expressions were determined by NanoString nCounter analysis (upper) and Q-PCR (lower) in samples from metastatic prostate cancer (PCa) (n=20) and localized PCa (n=20). β-actin was used as an internal control to normalize mRNA expression of CTAs.